

## Review Article

# The Emergence of Young Circulating Systemic Environment Factor as a Potential Strategy to Rejuvenate Biological Aging

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## Abstract

Aging results in degenerative and chronic inflammatory conditions, which are the risk factors for age-related cardiovascular, neurodegenerative, and cancer diseases. Therefore, it is of great importance to develop rejuvenation factors that promote regeneration and attenuate chronic inflammation in the elderly as a means to help control age-related diseases. Indeed, a promising “rejuvenation factor” present in young blood has been found by several scientific groups. Infusion of this youthful circulating systemic factor into aged individuals in mouse models can improve conditions of stem cell microenvironments in aged muscular, central nervous and myocardial systems, leading to rejuvenate stem cell ability to regenerate these systems. The identity of this factor could be circulating cytokines and chemokines (such as CCL11), growth factors (such as the transforming growth factor- $\beta$  superfamily member GDF11), or it could even be microRNAs that can epigenetically regulate gene expression. Whether this factor can beneficially impact the aged immune system and attenuate self-reactive T-lymphocyte derived chronic inflammatory conditions in the elderly is still under investigation. Understanding the characteristics and function of this factor holds great promise toward the development of novel therapeutics to reduce morbidity and mortality in age-related degenerative and inflammatory diseases.

**Keywords:** Aging; Circulating factor; Microenvironment; Rejuvenation; Inflamm-aging

## Introduction

It has been demonstrated that aging of certain systems can be reversed by providing old animals with young blood via the “heterochronic parabiosis” model, in which the circulating systems of young and aged mice are joined by surgery, or through infusion of young serum/plasma into old animals. Therefore, what constitutes young or old sera must be different. In order to explain these differences, the hypothesis of a “rejuvenation factor” in the young circulating system has been proposed. This factor has been demonstrated to improve the aged microenvironment, thereby promoting tissue-specific stem cell activation in most systems, including the skeletal muscle, central nervous and myocardial systems. The rejuvenation factor is defined as a “circulating systemic environmental factor” (CSEF). In this article, we briefly reviewed discovery of the CSEF, its possible identity, and potential applications in rejuvenation medicine, as well as outstanding questions for further studies.

### The “seed and soil” hypothesis is applied to stem cells and their niche during aging

Organismal aging is generally believed to result from a sharp decline in the regenerative capacity of stem cells and/or exhaustion of the stem cell pool [1,2]. However, stem cells are present and regulated by their surrounding microenvironment (stem cell niche) [3,4], which is comprised of stromal cells. Stem cells are similar to the

“seed”, while stromal cells around them are similar to the “soil”. There has been a long-standing argument over whether primary aging arises from stem cells *per se* or stem-cell niche cells [5,6]. Tissue-specific stem cells (the seeds) usually persist in a dormant state in adults. They will be aroused as needs for tissue homeostasis or injury repair. The signals to wake stem cells are provided by stem-cell niche cells (the soil). Mounting evidence indicates that age-induced defects primarily arise from the stem-cell niches, which are unable to provide appropriate signals or provide incorrect signals to the stem cells, rather than the stem cells *per se*. This ultimately leads to whole system failure. This hypothesis has been demonstrated in aged oocytes/ovary [7,8], sperm/testis [9,10], and muscles [11,12], as well as in the T-lymphoid system [13]. As should be expected, this viewpoint has attracted significant attention in recent years [6,14-16].

Based on this viewpoint, more and more studies are focusing on the development of strategies that aim to rejuvenate aged systems through improvement of the aged stem cell environment. For example, young blood serum has been used to rejuvenate the age-related decline of local stem-cell niches in order to improve progenitor cell activity [12]. The researchers proposed that there is certain “circulating systemic environmental factor”, or called CSEF, in the blood serum that changes with age. It is this CSEF from young serum that rejuvenates the activation of aged stem cells (such as satellite cells in muscle) via upregulation of *Notch* ligand Delta protein [12] and Wnt signaling [17].

Additionally, it has been shown that the CSEF can be exchanged in a “heterochronic parabiosis” model, in which young and aged mice are joined by surgery, which resulted in mutual influence via blood-borne factors [12,17-24]. Since the “heterochronic parabiosis” model consists of two living animals of different ages that are joined and develop a single shared circulatory system [12,17-19,23,24], the CSEF from both groups should influence each other. Evidence already showed that the CSEF from young mice can positively affect old mice, through which young CSEF enhances aged stem cell rejuvenation, while the CSEF from old mice can negatively influence young mice, through which old CSEF reduces neurogenesis [23] and worsens the immune system [18] in the young partners.

### Identity (protein and/or microRNA?) of the “CSEF” in serum/plasma and its regulatory pathway

Although the “CSEF” from young serum holds great promise as a rejuvenating medicine, its identity has been a puzzle for a long time. There must be multiple factors involved, such as the classic secreted factors: cytokine and chemokine, growth factors, angiogenic factors, and other circulating immunosystem components. Empirical evidence has shown that the CSEF is probably a type of protein or large peptide, which are sensitive to heat inactivation, because heated young plasma completely lost the beneficial effects that allowed raw young plasma to make the “old” central nervous system (CNS) “younger” [22]. Villeda *et al.* identified the circulating chemokine CCL11 as a type of CSEF that promoted neurogenesis and improved declined cognition in aged mice [23]. Additionally, Loffredo *et al.* identified the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily member GDF11 as another type of CSEF that can reduce age-related cardiac hypertrophy and even heart failure in aged mice [20]. Most recently, two publications further confirmed that GDF11 is a CSEF, which can be applied to rejuvenate age-related skeletal muscle dysfunction [25] and increase cerebrovascular proliferation and promote neurogenesis in the subventricular zone [21].

For mouse neurogenic rejuvenation, daily administration of recombination GDF11 (rGDF11) (0.1mg/kg mouse body weight) for 4 weeks of continuous injections (48 injections) is required to get a 50% increase in the volume of brain blood vessels [21]. However, administering a total of 8 injections of whole blood plasma (100ul per injection) in 24 days can significantly improve the cognitive function of aged mice [22]. The results imply that plasma is more efficient than GDF11 alone. Therefore, a GDF11 may be not the sole protein constituting the CSEF.

However, the CSEF has also been proposed to have a cellular identity. Using the “heterochronic parabiosis” model with induced spinal cord injury in the aged partner, Ruckh *et al.* demonstrated that macrophages from the young partner (based on green fluorescence protein “GFP” tag) entered the aged CNS to conduct the clearance of myelin debris and to rejuvenate neural re-myelination in the aged CNS [19]. The macrophages (and microglia in the CNS) possess two characteristic phenotypes - M1 and M2 [26,27]. They change their physiology in response to environmental stimulation. M1 macrophages possess phagocytic function (through classical activation); while M2 macrophages lay down extracellular matrix components in order to promote tissue injury healing (through alternative activation), and play a role in anti-inflammation and

immune regulation (termed regulatory macrophages) [28]. Later, the same group conducted elegant experiments showing the rejuvenating capability of macrophages and microglia in there-myelination of aged mice using the “heterochronic parabiotic systems” [29]. They found that during the aged CNS re-myelination under exposure to young circulating factors there was a switch from an M1-dominant response (pro-inflammation in the early stage) to an M2-dominant response (recovery in the late stage) [29].

If the CSEF is a protein, its expression should be potentially regulated by genetic and epigenetic events in the postnatal life. Ample evidence indicates that epigenetics modifications control or mediate the aging process [30-34], age-related inflammatory diseases [35,36], and gene expression (increased or decreased) [37]. This is a reasonable explanation as to why GDF11 was decreased in the serum and spleen of aged mice, examined by Loffredo *et al.* [20]. Epigenetic regulation includes three major mechanisms. In addition to DNA methylation and histone modifications, the non-coding microRNAs (miRNAs) can circulate in the serum and other body fluids [38,39]. Recently, circulating miRNAs, which are borne as cargo in exosomes (the extracellular vehicles [40]), are proposed to regulate neurodegenerative diseases [41], age-related disease (cardiovascular and neurodegenerative diseases) [42], and inflamm-aging [43] to mediate epigenetic exchange during aging [33]. Therefore, miRNAs and/or exosomes may constitute the CSEF, or they either regulate the CSEF directly or target the CSEF pathway.

Taken together, it appears there may be multiple blood-borne CSEF factors that have yet to be confirmed. These factors could be proteins (cytokines, chemokines, and growth factors) and/or RNAs. Caution should be taken not to regard GDF11 as the only factor in the CSEF, or the only factor capable of rejuvenating the aged individual. The blood from aged individuals, which reduces GDF11 in the serum [20], negatively affects neurogenesis and cognitive function in young individuals [23]. This cannot be simply explained as aged serum diluting GDF11 in young serum. It is very possible that miRNAs and other protein factors, which are able to be delivered through the blood, could target the GDF11 pathway in order to increase or decrease its expression.

### Potential applications of the young CSEF to make the aged milieu “younger”, i.e. improve the quality of the “soil”, in rejuvenating medicine

The young circulating systemic environmental factors hold great promise to improve the quality of the “soil” and are of great potential for rejuvenating medicine. Several independent research groups have already tested the use of young CSEF to rejuvenate several aged systems, such as muscular, myocardial, and central nervous systems.

By using the “heterochronic parabiosis”, in 2005, Conboy *et al.* found that young serum was able to rejuvenate the activation of aged muscle stem cells (satellite cells) via up-regulation of *Notch* ligand Delta protein and Wnt signal [12,17]. Recently, Sinha *et al.* found the same effect on muscle stem cells, and they also revealed that GDF-11 is the CSEF responsible for the rejuvenation of age-related skeletal muscle dysfunction [25]. Supplementation of rGDF-11 increased systemic GDF11 levels and restored genomic integrity and improved functional deficits in aged muscle stem cells, as well as improved muscle physiology and physical function. These results imply that

GDF-11 may be therapeutically useful for rejuvenating age-related skeletal muscle and stem cell dysfunction.

In myocardium rejuvenation, rGDF-11 could reverse cardiac hypertrophy in aged mice [20]. Using the surgical technique of the “heterochronic parabiosis”, the authors found that cardiac hypertrophy in aged mice significantly regressed after 4 weeks of exposure to young circulation. They then performed proteomics analysis and identified the circulating factor GDF-11 as being responsible for the rejuvenating effect on the heart. Injection of rGDF-11 in aged mice reversed cardiac hypertrophy and molecular remodeling.

Another study for rejuvenation of aged brain function using GDF11 was performed by Katsimpardi *et al.* [21]. They showed that 4 weeks of daily GDF11 injections worked nearly as well as the “heterochronic parabiosis” in improving proliferation of cerebral vasculature and promoting neurogenesis in the subventricular zone in the aged mouse brain [21]. Meanwhile, Villeda *et al.* also reported the same effect that injection of young serum can rejuvenate the aged brain hippocampus at the molecular, structural, functional and cognitive level [22]. In their research, they obtained the same effect as the “heterochronic parabiosis” by administration of young blood plasma 8 times over 24 days. This study also revealed that this hippocampal-dependent cognitive enhancement was partly mediated by cyclic AMP response element binding protein (Creb), which was activated by exposure to young blood factor [22]. All these findings in the rejuvenation of brain function by CSEF are of great clinical potential for Alzheimer patients and others suffering from age-related cognitive impairments [44,45].

### The potential role of the CSEF on the aged immune system

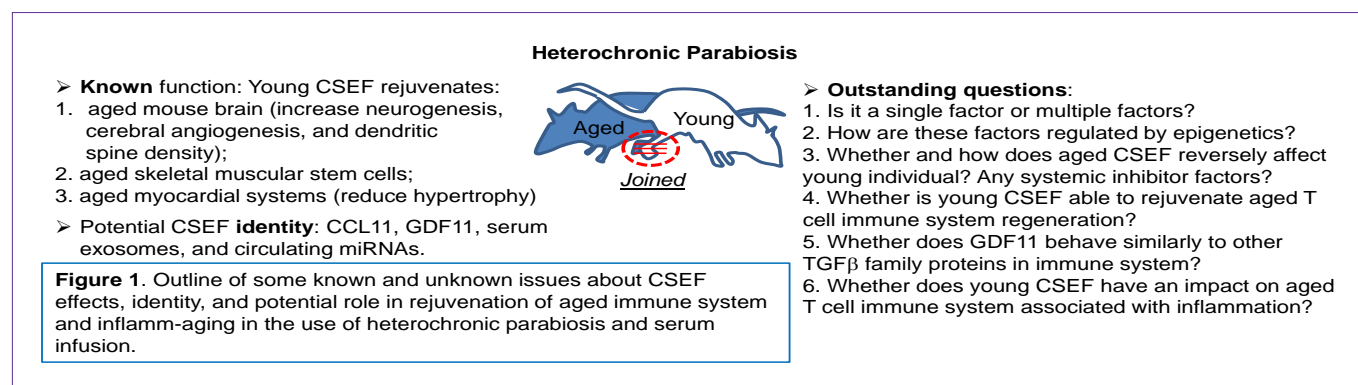
Applying the young CSEF to rejuvenate aged systems is a very promising strategy for interventional medicine, potentially able to cure or prevent many age-related diseases, including Alzheimer’s and heart disease. These age-related diseases are generally associated with age-related chronic inflammation (so called inflamm-aging) [46-50], i.e. persistent low-grade, but exceeding baseline levels of, pro-inflammatory factors in the elderly. In addition to CSEF’s direct function on rejuvenating aged muscular, myocardial, and central nervous systems [12,17,20-22], it is not known if CSEF can be applied to immune systems, to rejuvenate the profiles of an aged T cell pool and ameliorate immune cell participated chronic inflammatory conditions [46-51]. There was an uncertain result and insufficient evidence that young circulating factor could not restore the immune

system of the old partner and may even accelerate aging [18]. However, since GDF11 (potential CSEF) expression has been documented to be highly expressed in the immunological organs, including the spleen (the organ with highest expression) and the thymus (third highest expression), and the expression of GDF11 declines with age in these organs [20], it is reasonable to assume that GDF11 should impact the aged immune system. Since CSEF can be used for rejuvenating therapy for age-related changes to the CNS, muscle, and myocardium, it becomes increasingly important to determine how CSEF may affect the immune system: beneficial, detrimental, or otherwise. The work will help the rejuvenation of age-related risk factors to cardiovascular and neurodegenerative diseases [52,53].

Additionally, whether the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily member GDF11 behaves similarly to other TGF- $\beta$  family member proteins in the thymus and spleen is unknown. The function of other TGF- $\beta$  family proteins in immune aging and disease remains controversial. Using a conditional transforming growth factor- $\beta$  receptor-II (*tgfr2*) knockout mouse model with Cre driven by the *FoxN1* promoter, Hauri-Hohl *et al.* [54] showed a negative role of TGF- $\beta$  signaling in medullary thymic epithelial cells, which contributes to thymic involution and may lead to the survival of auto-reactive T cells. Conversely, using a similar mouse model, Ouyang *et al.* [55] demonstrated a positive role of TGF- $\beta$  signaling in regulating peripheral tolerance by aiding in the survival of natural regulatory T cells. The absence of TGF- $\beta$  signaling led to widespread activation of T cells and diabetes development. Therefore, it is necessary to know whether supplementation of rGDF-11 (GDF11, a TGF- $\beta$  family member) would be beneficial in reversing age-related immune system defects.

### Summary: Outstanding questions and potential applications of CSEF

Understanding the identity and regulatory pathway of CSEF will be essential in moving the therapeutic applications of CSEF forward. However, there may also be unrecognized issues. For example, it is unclear whether CSEF is comprised of only one factor, such as GDF11, or several factors in the circulating system? Even if GDF11 is the predominant or only factor making up the CSEF, how the GDF11 pathway is regulated by epigenetic regulators and how GDF11 improves aged tissue specific stem cell microenvironment are largely undetermined. Using the “heterochronic parabiosis”, in which young-old animal circulation systems are combined, most reports show young CSEF dominantly affects aged milieu, while a few reports





reveal aged CSEF also influences young milieu [18,23]. Is this just simply due to insufficiency of the CSEF in aged milieu? Is it possible that there are any systemic inhibitory factors with the aged CSEF that induces the young milieu to worsen? If there are systemic inhibitory factors, can we suppress them in aged individuals to rejuvenate systemic aging? Another important systemic that is drastically altered with age is the immune system, which increases the risk of age-related cardiovascular, neurodegenerative, and cancer diseases. However, whether the CSEF accelerates or rejuvenates immune system aging remains somewhat controversial [18]. Furthermore, the spleen and thymus can produce a large amount of GDF11 [20], since these organs are heterozygous organs constituted by at least two types of cells: lymphocytes and stromal cells, what cell type is the main GDF11 producer is not covered. These known and unknown aspects are briefly summarized in Figure 1 for further discussion. Although there are many more details to be unraveled, these studies indeed have made great progress toward rejuvenating aged systems and these clues could be used to develop new and much-needed therapeutic strategies to reduce the risk of age-related degenerative and age-related inflammatory diseases.

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